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EXAMINER	
RAMIREZ, DELIA M	

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1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/720,177	NAKAMURA ET AL.	
	Examiner	Art Unit	
	DELIA M. RAMIREZ	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 August 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,4,5,8-16 and 18-21* is/are pending in the application.

4a) Of the above claim(s) *8-11* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,4,5,12-16 and 18-21* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *25 November 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *alignments*.

DETAILED ACTION

Status of the Application

Claims 1, 4-5, 8-16, 18-21 are pending.

The instant Office action is in response to the panel decision from pre-appeal brief review mailed on 8/4/2008. The finality of the previous Office action mailed on 3/17/2008 is withdrawn as new grounds of rejection and/or new references are hereby introduced. An action on the merits follows.

Claims 8-11 remain withdrawn from consideration as being directed to an invention non-elected with traverse in a communication filed on 4/25/2005. Since no product claim is allowable at this time, a restriction requirement between product and process claims can be properly maintained. Claims 1, 4-5, 12-16, 18-21 are at issue and are being examined herein.

Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Rejections - 35 USC § 112, Second Paragraph

1. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

2. Claims 1, 4-5, 12-16, 18-21 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

3. This rejection has been discussed at length in the previous Office action mailed on 3/17/2008. It is maintained for the reasons of record and those set forth below. It should be noted that in the previous Office action the Examiner inadvertently omitted to include claim 12 in the list of rejected claims. Thus, with regard to claim 12, the instant rejection is a new rejection not previously introduced.

4. Claims 1, 5 and 16 (claims 4, 12-15, 18-21 dependent thereon) are indefinite in the recitation of "95% or more homologous to SEQ ID NO: X".

5. Applicant argues in the Pre-Appeal Brief filed on 6/17/2008 that the term "95% or more homologous to SEQ ID NO: X" is universally accepted as being clear and definite in view of the standardized and well-practiced methods for determining homology between sequences known in the art. Applicant refers to programs such as BLAST in support of the argument that methods to calculate homology are well known in the art and that the parameters used in that program have been standardized so that homology determination is commonly and routinely practiced in the art with consistent and meaningful results.

6. Applicant's arguments have been fully considered but are not deemed persuasive to overcome the instant rejection. It should be noted that claims 12 and 18 have been included in the instant rejection because these claims are dependent upon claim 1 and as such the genus of glutaminase genes recited does not encompass all possible genes encoding the polypeptide of SEQ ID NO: 2 but rather only those genes which encode the polypeptide of SEQ ID NO: 2 and meet the structural limitations of the gene as set forth in items (a) or (b) of claim 1. In other words, the genus of genes recited in claims 12 and 18 are a subgroup of the entire group of genes encoding the polypeptide of SEQ ID NO: 2.

The Examiner acknowledges that the art teaches several methods to calculate homology between sequences. The examiner also acknowledges that there are some parameters used in BLAST which are considered default parameters and are preferred in the calculation of homology. However, the issue in this case is not whether one of skill in the art would not know how to calculate homology, or whether one of skill in the art would not recognize that there are some preferred methods of calculation and preferred parameter values in those preferred methods of calculation. The issue in the instant case is whether one of skill in the art can determine the scope of the claims when the numerical value recited can be calculated in different ways and neither the claim nor the specification provides a clue as to which is the intended method to calculate such value so that it would be clear as to which species fall within the genus of nucleic acids recited and which ones are not encompassed by the genus recited.

It is reiterated herein that the term is unclear and confusing in the absence of a definition providing the intended meaning of the term or the intended parameters required to determine the required homology value. As previously stated, while one of skill in the art can interpret the term "homology" as "identity", these terms are not equivalent. The calculation of sequence homology takes into consideration the type of mismatches such that even mismatches contribute to the % homology value, whereas mismatches are given no weight when calculating the % identity value, i.e., only matches contribute to the % identity value. Since (1) there is no indication in the specification that the intended meaning of the term "homology" is "identity", (2) it is clear from Applicant's response that the intended meaning of the term is not identity, and (3) the specification does not provide the specific parameters/methods intended in the calculation of sequence homology (e.g., PAM matrices), one of skill in the art cannot determine the scope of the term "95% homologous" because a percent sequence homology value is variable depending on the parameters used in the calculation and Applicant has not set forth the intended method/parameters for that calculation. One could have a nucleic acid sequence which is 95% sequence homologous to a reference sequence based on a particular set of parameters/matrix, and at the same time that same nucleic acid sequence being not 95% sequence homologous to the reference sequence if another set of parameters/matrix is used in the calculation. For example, using the scoring table (matrix) IDENTITY_NUC and parameter values Gapop = 10.0 and Gapext = 1.0, the polynucleotide of GenBank accession number BA000036_26 is 95.4 % sequence homologous to the polynucleotide of SEQ ID NO: 1. If the same calculation is carried out with the same scoring table and parameter values Gapop = 10.0 and Gapext = 0.1, the polynucleotide of GenBank accession number BA000036_26 is 96.1 % sequence homologous to the polynucleotide of SEQ ID NO: 1. See attached alignments (Scoring Table and parameters shown at the first page; homology is indicated as Query Match immediately prior to the alignment). If the claims were to recite "96% or more homologous to SEQ ID NO: 1", would the polynucleotide of GenBank accession number BA000036_26 meet the limitation of "96% or more"? The

answer to that question is unclear because it depends on how the calculation was made. In the instant case, the claims include a numerical limitation without disclosing which of the multiple methods of measuring that number should be used. As such, the term is deemed indefinite. In view of the fact that one of skill in the art cannot reasonably determine which species are encompassed by the claims, in the interest of advancing prosecution, no patentable weight will be given to the term "95% or more homologous to SEQ ID NO: 1". Correction is required.

Claim Rejections - 35 USC § 112, First Paragraph

7. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

8. Claims 1, 4-5, 12-16, 18-19 and 21 remain rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

9. This rejection has been discussed at length in the Final action mailed on 3/17/2008 and the Non Final action mailed on 3/5/2007. It is maintained for the reasons of record and those set forth below.

10. Applicant argues in the Pre-Appeal Brief filed on 6/17/2008 that from the alignment provided as Exhibit A in the response of 7/31/2007 one could clearly recognize which regions are important for enzymatic activity so that modifications could be made to reduce enzymatic activity. Similarly, Applicant argues that one could look at the regulatory regions of the glutaminase gene disclosed and determine similar regulatory regions on the chromosome of a coryneform bacterium so that modifications could be made that would disrupt the expression of the recited glutaminase gene. Applicant argues that with this knowledge, one of skill in the art would easily be able to obtain a coryneform bacterium having 0.1 U/mg or less glutaminase activity. With regard to glutamine synthetase genes, Applicant argues that in view of

the alignment provided as Exhibit B in the response of 7/31/2007, one of skill in the art would understand that a DNA which is more than 95% sequence homologous to the polynucleotide of SEQ ID NO: 3 would naturally encode a protein having glutamine synthetase activity. Applicant argues that claims 5 and 16 have been amended to restrict the means for increasing glutamine synthase activity.

11. Applicant's arguments have been fully considered but are not deemed persuasive to overcome the instant rejection. For the record, it is noted that in making the instant rejection, the Examiner has not considered the issue of indefiniteness due to the recitation of "95% or more homologous to SEQ ID NO: 1". It is reiterated herein that the Examiner acknowledges the amendments made and the limitations recited in the claims. However, as previously indicated, the written description issue regarding these claims is the fact that the claims require a precise reduction in glutaminase activity to a specific level of glutaminase activity (0.1 U/mg or 0.01U/mg) and a precise ratio of glutamine synthetase activity to glutaminase activity (2:1) obtained by any means. The Examiner agrees that one could disrupt a gene by introducing deletions/insertions within the coding region of a gene, or that one could disrupt the expression of a gene by deleting the regulatory region of a gene such that the protein encoded by that gene is completely inactive (0 Units/mg) or has some residual activity. The Examiner also agrees that one could enhance the activity of an enzyme by increasing the copy number of the gene encoding said enzyme, or by placing the gene encoding said enzyme under the control of a strong heterologous promoter such that one could increase the amount of that enzyme with respect to other proteins. However, in view of the fact that the claims require a precise level of reduction in enzymatic activity, the specification should provide some description as to how one of skill in the art should mutate the recited glutaminase genes such that those mutations would result in that precise level of enzymatic activity reduction in any coryneform bacterium as recited, or how should one modulate expression of the recited genes to obtain the desired level of enzymatic activity. Similarly, in view of the fact that the claims require a precise ratio of glutamine synthetase to glutaminase activity, the specification should provide

adequate description as to the modifications that can be made to a coryneform bacterium as recited such that one could achieve the required ratio. In addition, it should be noted some of the claims are not limited to a particular glutamine synthetase activity. Therefore, these claims encompass a coryneform bacterium where a gene encoding glutamine synthetase activity from any organism is modified in any way to increase glutamine synthetase activity such that the recited ratio is achieved.

With regard to the alignments provided in a previous response (Exhibits A and B), it is unclear to the Examiner as to how one could look at the conserved regions shown in the alignments and determine which modifications would result in the desired effect if (1) there is no structure/function correlation that would provide one of skill in the art with some suggestion as to the effect of making a particular modification on function, (2) the art as previously discussed clearly teaches the unpredictability of determining a priori the effect of structural changes on a protein's function based solely on structural similarity, and (3) the conserved regions shown in the alignment are the regions which are conserved among the proteins used in the alignment and there is no indication as to how these conserved regions are related to the enzymatic activity required by the claims.

With regard to arguments that the alignment provided would allow one of skill in the art to recognize that a DNA having the recited sequence homology to the polynucleotide of SEQ ID NO: 3 would naturally encode a glutamine synthase, it is noted that the art, as evidenced by Witkowski et al. (Biochemistry 38:11643-11650, 1999), teaches that even a single conservative substitution can result in enzymatic activity changes. Therefore, unless the art teaches that any DNA having the recited % homology to the polynucleotide of SEQ ID NO: 3 would encode a glutamine synthase, it is unlikely that one of skill in the art would reasonably conclude that any homolog of the polynucleotide of SEQ ID NO: 3 having the recited homology would necessarily encode a glutamine synthetase as asserted by Applicant. Thus, for the reasons of record and those forth above, one cannot reasonably conclude that the claimed invention is adequately described.

12. Claims 1, 4-5, 12-16, 18-21 remain rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a *C. glutamicum* cell wherein said cell has been modified to reduce the endogenous glutaminase activity and to increase glutamine synthetase activity, wherein the reduction in glutaminase activity is due to an inactivating deletion in the endogenous glutaminase gene of said *C. glutamicum* cell, wherein the endogenous glutaminase gene comprises SEQ ID NO: 1 prior to the introduction of the deletion, and the increase in glutamine synthase activity is due to (i) an increase in the copy number of the *C. glutamicum* glnA gene of SEQ ID NO: 3, or (ii) an increase in expression of the *C. glutamicum* glnA gene of SEQ ID NO: 3 by placing said gene under the control of a heterologous promoter, does not reasonably provide enablement for (1) a coryneform bacterium modified to reduce glutaminase activity to less than 0.1 U/mg protein or 0.01 U/mg protein in said bacterium by mutating any region of a glutaminase gene comprising SEQ ID NO: 1 or a structural homolog thereof, (2) the coryneform bacterium of (1) further modified in any way to increase any glutamine synthetase activity such that the recited ratio (2 to 1) of glutamine synthetase activity to glutaminase activity is achieved, or (3) the coryneform bacterium of (1) further modified by increasing the expression of a glutamine synthetase gene which is a structural homolog of the nucleic acid of SEQ ID NO: 3, wherein said increase in expression is obtained by increasing the copy number of the glutamine synthetase gene or by replacing the endogenous promoter of the glutamine synthetase gene with a stronger promoter. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

13. This rejection has been discussed at length in the Final action mailed on 3/17/2008 and the Non Final action mailed on 3/5/2007. It is maintained for the reasons of record and those set forth below.

14. Applicant's arguments as set forth in the Pre-Appeal Brief filed on 6/17/2008 regarding the 35 USC 112, first paragraph rejections have been summarized above.

15. Applicant's arguments have been fully considered but are not deemed persuasive to overcome the instant rejection. As indicated above, the Examiner acknowledges the amendments made, the teachings of the specification and the art, as well as the limitations recited in the claims. However, the Examiner disagrees with Applicant's contention that the specification and the teachings of the prior art would enable the entire scope of the claims. The instant rejection has been made in view of the lack of information in the specification and the prior art as to (1) the modifications required to obtain the precise ratio of glutamine synthase to glutaminase activity recited by the claims, (2) the mutations in the entire genus of glutaminase genes recited which would lead to the precise reduction in glutaminase activity recited, and (3) how to isolate the entire genus of glutamine synthetase genes recited in the claims. It is reiterated herein that while it is agreed that one could disrupt a gene by introducing deletions/insertions within the coding region of a gene, or that one could disrupt the expression of a gene by deleting the regulatory region of a gene such that the protein encoded by that gene is completely inactive (0 Units/mg) or has some residual activity, the specification is silent with regard to the mutations that can be made to the recited genes to obtain the precise level of reduction in glutaminase activity recited in the claims. Similarly, while it is agreed that one could enhance the activity of an enzyme by increasing the copy number of the gene encoding said enzyme, or by placing the gene encoding said enzyme under the control of a strong heterologous promoter such that one could increase the amount of that enzyme with respect to other proteins, the specification is silent with regard to the modifications which would result in the specific ratio recited, or how to modulate expression with the methods for increasing enzymatic activity described above such that the ratio recited is achieved. As indicated in previous actions, the mutations/modifications required by the claims encompass more than those described in the specification and include, for example, addition of unknown transcription inducers, expression of unknown proteins which would alter the transcription of the recited genes, unknown mutations in the regulatory regions of the recited genes to increase/decrease expression, and unknown mutations within the coding regions of

the recited genes to alter the enzymatic activities recited so that the recited reduction levels or ratio is obtained.

While Applicant argues that one could look at the conserved and variable regions shown in the alignments and determine which modifications would result in the desired effect, it is noted that (1) there is no structure/function correlation that would provide one of skill in the art with some suggestion as to the effect of making a particular modification on function, (2) the art as previously discussed clearly teaches the unpredictability of determining a priori the effect of structural changes on a protein's function based solely on structural similarity, and (3) the conserved regions shown in the alignment are the regions which are conserved among the proteins used in the alignment and there is no indication as to how these conserved regions are related to the enzymatic activity required by the claims. Thus, contrary to Applicant's assertion, alignments such as those provided by Applicant would not provide the necessary structure/function correlation which would allow one of skill in the art to determine a priori which modifications are most likely to result in the desired effect. Therefore, to enable the entire scope of the claims, one of skill in the art would have to test an infinite number of mutations to determine which ones result in the desired enzymatic activity, and test an extremely large number of modifications to determine which ones result in the desired ratio of glutamine synthetase to glutaminase.

With regard to the genus of glutamine synthetase genes encompassed by the claims, even if one were to assume that the term "95% or more homologous to SEQ ID NO: 1" is equivalent to "95% or more sequence identical to the polynucleotide of SEQ ID NO: 1", the genus of polynucleotides to be tested for glutamine synthetase activity is immense. As indicated in the action mailed on 3/5/2007, the total number of variants of a polynucleotide having a specific sequence identity can be calculated from the formula $N!x3^{A}/(N-A)!/A!$, where N is the length in nucleotides of the reference polynucleotide and A is the number of allowed substitutions for a specific % identity. Thus, for a variant of the polynucleotide of SEQ ID NO: 3 having 95% sequence identity to the polynucleotide of SEQ ID NO: 3, the total

number of variants to be tested is 2500!x3^{125}/(2500-125)!/125! (SEQ ID NO: 3 has 2500 nucleotides; 125 = 0.05x2500) or 5.3x10^{273} variants. While Applicant argues that one of skill in the art would recognize that a DNA having the recited sequence homology to the polynucleotide of SEQ ID NO: 3 would naturally encode a glutamine synthase, it is noted that the art, as evidenced by Witkowski et al. (Biochemistry 38:11643-11650, 1999), teaches that even a single conservative substitution can result in enzymatic activity changes. Therefore, unless the art teaches that any DNA having, for example, 95% sequence identity to the polynucleotide of SEQ ID NO: 3 would encode a glutamine synthase, it is unlikely that one of skill in the art would reasonably conclude that all 5.3x10^{273} variants of the polynucleotide of SEQ ID NO: 3 having 95% sequence identity to the polynucleotide of SEQ ID NO: 3 would necessarily encode a glutamine synthetase as asserted by Applicant. Since no correlation between structure and function has been provided, no disclosure of the structural features characteristic of polynucleotides encoding glutamine synthetases has been provided, and no rational scheme has been provided to limit the number of species to be tested to a reasonable amount, one of skill in the art would have to test an essentially infinite number of polynucleotides to determine which ones have glutamine synthetase activity. Thus, one cannot reasonably conclude that the entire scope of the claimed invention is enabled by the teachings of the specification and/or the prior art.

Claim Rejections - 35 USC § 103

16. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

17. Claims 1, 4-5, 13-16, 19-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nakamura et al. (EP 1229121 A2 published 8/7/2002; cited in the IDS) in view of Pompejus et al. (WO 01/00843, published 1/4/2001; cited in the IDS), Jakoby et al. (GenBank accession number Y13221, 1997; FEMS Microbiol. Lett. 154(1):81-88, 1997; both cited in the IDS), and further in view of Duran et

al. (Microbiology 141:2883-2889, 1995), as evidenced by Nakagawa et al. (GenBank accession number AX127151, 2001; EP 1 108 790 published 6/20/2001).

18. The basis for this rejection has been extensively discussed in previous Office actions. However, additional references which further support this rejection are hereby introduced. To that extent, the instant rejection introduces new grounds of rejection.

19. Nakamura et al. teach a method for producing L-glutamine by fermentation of an L-glutamine producing *C. glutamicum* cell, wherein said cell has been modified to increase the intracellular concentration of glutamine synthetase by increasing the copy number of the glnA gene of *C. glutamicum* (encodes glutamine synthetase; Example 1, Table 1, strain AJ12418/pGS). Nakamura et al. also teach a method for production of L-glutamine and suppression of L-glutamic acid as a by-product (paragraph [005]-[006]). Nakamura et al. do not teach a method for producing L-glutamine wherein glutaminase activity is reduced.

Duran et al. teach that glutaminase degrades glutamine to yield glutamate and ammonium (page 2884, left column, first full paragraph) and disclose a mutant *R. etli* (LM16) wherein the chromosomal glutaminase gene is disrupted by Tn5 mutagenesis (Page 2884, Methods, Strains and plasmid). The reference also teaches that LM16 produces more glutamine than glutamate when cultured with different substrates (page 2886, Table 1). As shown in Table 1, the amount of glutamine produced varies from 53X (49/0.9) to 2X (0.8/04) more glutamine in the glutaminase deficient mutant LM16 as compared to the wild type *R. etli*. Duran et al. do not teach a *C. glutamicum* or coryneform bacterium deficient in glutaminase.

Jakoby et al. teach a *C. glutamicum* glutamine synthetase gene and its corresponding glutamine synthetase. The gene of Jacoby et al. is 99.1% sequence identical to the polynucleotide of SEQ ID NO: 3 (99.1% = 2479x100/2500). See attached alignment. Jakoby et al. do not teach a method for producing L-glutamine in coryneform bacteria wherein glutaminase activity is reduced.

Pompejus et al. teach a *C. glutamicum* strain and a gene from that *C. glutamicum* strain encoding glutaminase (Table 1, page 56, Glutamate and Glutamine metabolism, RXA00335 and RXN03176; SEQ ID NO: 101-102). The nucleotide sequence of the glutaminase gene of Pompejus et al. has been disclosed as SEQ ID NO: 101 in Pompejus et al. and is 861 nucleotides long. An alignment of this polynucleotide with the polynucleotide of SEQ ID NO: 1 shows that this sequence is 99% sequence identical to nucleotides 827-1687 of SEQ ID NO: 1 (99% = 851x100/861; see previously provided alignment). In view of the fact that the specification discloses that the gene encoding the *C. glutamicum* glutaminase of SEQ ID NO: 2 is 2100 nucleotides long, one would reasonably expect the glutaminase gene in the *C. glutamicum* of Pompejus et al. to have a nucleotide sequence which is longer that that disclosed originally by Pompejus et al. (longer than SEQ ID NO: 101 of Pompejus et al.) by virtue of being from the same organism, i.e.., *C. glutamicum*, and at the same locus since the percent identity of SEQ ID NO: 101 with a fragment of SEQ ID NO: 1 is extremely high (99%). While Pompejus et al. do not disclose the entire sequence of their glutaminase gene, Nakagawa et al. provides the remaining structure of the *C. glutamicum* glutaminase (GenBank accession number AX127151) gene of Pompejus et al. This is shown in an alignment of SEQ ID NO: 101 from Pompejus et al. against chromosomal DNA from *C. glutamicum* taught by Nakagawa et al. (GenBank accession number AX127151) where SEQ ID NO: 101 from Pompejus et al. is completely comprised by the chromosomal DNA taught by Nakagawa et al. If one aligns the chromosomal DNA fragment of Nakagawa et al. that comprises the entire sequence of the *C. glutamicum* glutaminase gene of Pompejus et al., it is shown that the *C. glutamicum* of Pompejus et al. comprises a glutaminase gene which is 98.0% sequence identical to the polynucleotide of SEQ ID NO: 1 (98.0% = 2059x100/2100). See attached alignments. Therefore, even if one were to interpret the term "95% or more homologous to SEQ ID NO: 1/3" as "95% or more sequence identical to the polynucleotide of SEQ ID NO: 1/3, (1) the *C. glutamicum* of Pompejus et al., as evidenced by Nakagawa et al., comprises an endogenous glutaminase gene which is more than 95% sequence identical to the

polynucleotide of SEQ ID NO: 1, and (2) the glutamine synthetase gene of Jakoby et al. is at least 95% sequence identical to the polynucleotide of SEQ ID NO: 3. Pompejus et al. also teach that the disclosed *C. glutamicum* gene can be used for the modulation of production of amino acids (page 11, lines 20-25) and that glutamine is used in both pharmaceutical and cosmetics industries (page 13, lines 17-19). Pompejus et al. do not teach a mutant coryneform bacterium wherein the glutaminase activity in said bacterium has been reduced and glutamine synthetase activity has been enhanced.

Claims 1, 4-5, 13-16, 19-21 are directed in part to a coryneform bacterium that produces L-glutamine modified such that (1) the glutaminase activity of said bacterium is reduced by disrupting the endogenous glutaminase gene on the chromosome, and the glutamine synthetase activity in said bacterium is increased by increasing the copy number of a gene encoding a glutamine synthetase or by placing said gene under the control of the lac, trp, or tre promoter, wherein the glutaminase gene to be disrupted hybridizes under the stringent conditions recited in claim 1 to the polynucleotide of SEQ ID NO: 1 and the glutamine synthetase gene hybridizes under the stringent conditions recited in claims 5 and 16 to the polynucleotide of SEQ ID NO: 3, wherein the glutamine synthetase activity in said bacterium is at least double that of the glutaminase activity, and wherein said glutaminase activity is 0.01 U/mg protein or less. See Claim Rejections under 35 USC 112, second paragraph, for claim interpretation.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the *C. glutamicum* cell that endogenously comprises the *C. glutamicum* glutaminase gene of Pompejus et al. by (1) deleting all or most of the coding region of the glutaminase gene, and (2) increasing the expression of the *C. glutamicum* glutamine synthetase gene of Jakoby et al. either by increasing its copy number or by placing said gene under the control of the lac, trp, or tre promoters. A person of ordinary skill in the art is motivated to construct such *C. glutamicum* cell in view of the fact that (1) Duran et al. teach an increase in L-glutamine production when the glutaminase gene is disrupted, (2) Pompejus et al. teach that L-glutamine is a chemical used in the pharmaceutical and cosmetics industries,

(3) Duran et al. teach that glutaminase degrades L-glutamine to glutamate, (4) Nakamura et al. teach a method for the production of L-glutamine where a reduction in the production of L-glutamic acid is desired, (5) Nakamura et al. teach that increasing glutamine synthetase activity results in an increase in L-glutamine production, and (6) the use of strong heterologous promoters allows for controlled expression of the protein of interest as they require the presence of inducers for expression to occur (e.g., lactose and tryptophan).

One of ordinary skill in the art has a reasonable expectation of success at modifying such *C. glutamicum* cell in view of the fact that Pompejus et al. teach the *C. glutamicum* comprising the endogenous glutaminase gene, Jakoby et al. teach the *C. glutamicum* glutamine synthetase gene, and inactivation of genes by deletion if the sequence of the target gene is known is well known and widely practiced in the art, Nakamura et al. teach increased expression of the glutamine synthetase gene for increased L-glutamine production in *C. glutamicum*, Duran et al. teach that inactivation of the glutaminase gene results in increased L-glutamine production, and increased expression by increasing the copy number of the gene of interest and the use of lac, trc, or trp promoters is well known in the art. In the absence evidence to the contrary, if no additional sources of glutaminase activity are present, a deletion of the glutaminase gene wherein most or all of the coding region is removed would result in no glutaminase activity (i.e., 0 U/mg protein). If the glutaminase activity is 0 U/mg protein, then the glutamine synthetase activity would be expected to be at least double that of glutaminase. Therefore, the invention as a whole would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made.

20. Applicant reiterates the arguments presented in the response of 7/31/2007. Applicant argues that the strain of Duran et al. is not a glutamine-producing strain because the strain of Duran et al., when grown in the presence of succinate and glutamine, displays intracellular levels of glutamine which are lower than when the strain is grown solely in the presence of glutamine. Applicant submits that the strain

of Duran et al. is known to have an additional enzyme (called GOGAT by Applicant; known in the art as glutamate synthetase) which can also degrade glutamine in the presence of α–ketoglutarate. Applicant suggests that the reason why Duran et al. observed an accumulation of glutamine was not due to the inactivation of the glutaminase gene but due to the low levels of α–ketoglutarate, which did not allow GOGAT to degrade glutamine. Applicant argues that when succinate was added, less glutamine was accumulated, probably because the addition of succinate allowed for the formation of α–ketoglutarate, which in turn allowed GOGAT to degrade glutamine. Applicant speculates that if glucose would have been added to the medium, GOGAT would have degraded glutamine even more. Applicant concludes that the teachings of Duran et al. do not teach or suggest that an increase in glutamine levels in a glutaminase-deficient strain when glucose is present in the medium. With regard to Nakamura et al., Applicant submits that Nakamura et al. teach glutamine fermentation by a coryneform bacterium in the presence of glucose and does not teach disabling the glutaminase gene. With regard to Pompejus et al., while not disputing the fact that this reference teaches the glutaminase and glutamine synthetase genes from *C. glutamicum*, Applicant submits that there is no reason for one of skill in the art to combine the teachings of these references.

21. Applicant's arguments have been fully considered but are not deemed persuasive to overcome the instant rejection or avoid the new grounds of rejection introduced in the instant Office action. As previously indicated claims 20-21 are directed in part to the bacterium of claim 1 with the added limitation that the glutaminase gene in the chromosome has been disrupted. As known in the art, mutating a gene on the chromosome can encompass disrupting that gene via a deletion in that gene. Also, while the Examiner has not given any patentable weight to the term "95% or more homologous to SEQ ID NO: X" for the reasons extensively discussed above in Claim Rejections under 35 USC 112, second paragraph, even if the term were to be interpreted as "95% or more sequence homologous to the

polynucleotide of SEQ ID NO: X", the nucleic acids of Pompejus et al. and Jakoby et al. would meet this limitation. See above for detailed explanation.

It is reiterated herein that it is agreed that degradation of glutamine is not carried out only by glutaminase since glutamate synthetase would also use glutamine as a substrate. However, even if one takes into account the possibility of other enzymes also degrading glutamine, this fact would not lead one of skill in the art to conclude that it is unlikely that inactivating glutaminase would not result in some reduction in glutamine degradation. While Duran et al. teach that growth substrate is a factor in how much glutamine accumulation is obtained, even when peptone+yeast extract was used, Duran et al. observed twice the amount of intracellular glutamine compared to the wild type strain (Table 1, last entry). If the objective is to produce glutamine, the results of Duran et al. would not only provide motivation to create a coryneform bacterium which is glutaminase-deficient for enhanced glutamine production, but would also provide the skilled artisan with a reasonable expectation of some glutamine accumulation as a result of inactivating glutaminase.

As previously stated, the Examiner disagrees with the argument that Duran et al. teach away from using a glutaminase-deficient strain for accumulation of glutamine if the strain is grown in the presence of glucose, in view of the fact that (1) there is no teaching or suggestion in Duran et al. indicating that no glutamine accumulation is expected in the presence of glucose, and (2) there is no experimental evidence by Applicant or the prior art showing that the strain of Duran et al., when grown in the presence of glucose, would provide absolutely no glutamine accumulation. Even if it is assumed that the contribution of GOGAT in glutamine degradation would be higher if the strain is grown in glucose, it is unlikely that the lack of glutaminase activity would have no effect whatsoever on glutamine levels. The teachings of Duran et al. clearly suggest that there is an effect on glutamine levels when glutaminase is inactivated. Therefore, the teachings of the art, as evidenced by Duran et al., make the inactivation of glutaminase not only obvious to try but also provide a reasonable expectation of observing some glutamine accumulation.

With regard to arguments that Duran et al. do not teach an L-glutamine producing organism, it is noted that while it is acknowledged that the strain of Duran et al. is not considered a high L-glutamine producer such as the *C. glutamicum* strain taught by Pompejus et al. or Nakamura et al., it meets the definition given in the specification for an L-glutamine producing organism in view of the fact that this bacterium will produce L-glutamine and some L-glutamine will accumulate in the growth medium partly due to cell lysis. It should be noted that the definition provided in the specification does not place any limitations on how much L-glutamine has to accumulate in the medium for the organism to be considered an L-glutamine producer. As indicated above and in previous Office actions, the Examiner has not relied on Duran et al. for meeting the limitation "coryneform bacterium having L-glutamine-producing ability" as required by the claims.

With regard to the teachings of Nakamura et al., it is noted that Nakamura et al. do not teach glucose-containing media as the only media in which coryneform bacteria can be grown, nor does it teach away from growing *C. glutamicum* in media that do not contain glucose. Also, while it is acknowledged that Nakamura et al. do not teach inactivation of the glutaminase gene, this limitation is clearly suggested by the teachings of Duran et al. previously discussed. Thus, contrary to Applicant's assertions, there is not only a clear motivation but a reasonable expectation of success in combining the teachings of Nakamura et al., Duran et al., and Pompejus et al. As such, the claimed invention is deemed obvious over the cited prior art.

Conclusion

22. No claim is in condition for allowance.

23. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PMR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through

Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

24. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Delia M. Ramirez whose telephone number is (571) 272-0938. The examiner can normally be reached on Monday-Friday from 8:30 AM to 5:00 PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Nashaat Nashed can be reached on (571) 272-0934. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (571) 272-1600.

/Delia M. Ramirez/

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DR
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